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Some Reactions of the Blood after Cancer Inoculation

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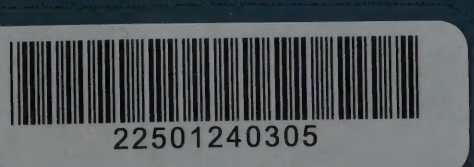

Cancer
D/F

SECTION V

THERAPEUTICS

INDEPENDENT PAPER

SOME REACTIONS OF THE BLOOD AFTER CANCER INOCULATION

By J. A. SHAW-MACKENZIE, M.D., London

The opportunity of investigating the blood conditions in carcinoma and on the chemical side of the cancer problem was afforded me by Professor W. D. Halliburton, in the Physiological Laboratory of King's College, London. Whilst working there, Dr. Otto Rosenheim suggested that lipase, the fat-splitting enzyme of the pancreas, should be taken into account also, and partly in conjunction with him I published preliminary accounts of the results obtained.[1]

It was shown that serum increases the activity of pancreatic lipase—an observation made originally by Pottevin,[2] working with horse serum.

In certain cases of carcinoma in the human subject and in other pathological conditions, however, it was found that this reaction of the serum was considerably increased.

Rosenheim[3] found that the lipase contained in glycerin extracts of the pancreas can be separated by filtration into two inactive portions. On mixing together the residue on the filter and the filtrate the mixture exerts the same fat-splitting action as the unfiltered extract. The residue on the filter (*inactive lipase*) is destroyed by heat, but the other component, namely that in the filtrate, is thermostable: this second substance is termed the *co-enzyme* of pancreatic lipase. The activating effect of carcinomatous serum and serous fluids, whether boiled or unboiled, on this inactive lipase and on inactive fresh pancreatic juice is very pronounced and greater than that of the co-enzyme or of normal serum.

This property of the serum in pathological conditions was a new observation, so far as I know, and in some seventy-five cases of malignant disease, including sarcoma, rodent ulcer, and serous fluids—ascitic, pleuritic, and œdema fluid—since examined, I have found (1) the accelerating fat-splitting

[1] *Proceedings of the Physiological Society*, February, 1910; *Journal of Physiology*, 1910, xl, pp. viii and xii; also *Proc. Roy. Soc. Med.*, 1910 (Path. Sect.), iii. 168.

[2] H. Pottevin, *Comptes rend. Acad. Sci.*, 1903, cxxxvi. 767.

[3] O. Rosenheim, *Proceedings of the Physiological Society*, February, 1910; *Journal of Physiology*, 1910, xl, p. xiv.

reaction present in more or less degree. In addition the antitryptic power of the serum and of serous fluids in malignant disease was found to be increased in accordance with the original observations of Marcus [1], Brieger and Trebing [2]. (2) In progressive and advanced cases the lipoclastic acceleration is low, while the antitryptic action is greatly increased. (3) After apparent recovery, or improvement, the acceleration remains high or is increased. But under the same conditions the antitryptic value falls to normal, or may be sub-normal.[3]

In non-malignant cases investigation has not been made fully, so far, but there is no doubt that the accelerating action is present also in certain of these conditions in more or less degree. The reaction, therefore, is not specific for cancer. In such cases, however, so far examined and in animals whose serum shows a high power of accelerating fat-splitting, the antitryptic value is not greater than in normal serum.

I found that the accelerating action on fat-splitting is very high in the serum of (*a*) mice inoculated with mouse tumour; (*b*) mice which had proved 'negative' to inoculation; and (*c*) mice which had recovered spontaneously from large growths.[4] This was found also in mice inoculated with normal mouse tissues, and after injection with certain tissue extracts and substances.

It seemed to me therefore that this property of the serum was not a mere accident but may be an important factor in the natural or induced defensive and protective processes of the body. It served also to direct attention anew to treatment in inoperable cancer by the administration of bile salts, which are well-known coadjutors in the action of pancreatic lipase, and to subcutaneous injections of the patient's own serum or serous fluids [5]—auto-serum therapy.

I obtained similar reactions of the serum in inoculation of mouse tumour in the rat—that is, inoculation of cancer of a strange species. Under these conditions it is known that the grafts proliferate for a short time and then degenerate, and I found that the accelerating action of the serum on pancreatic lipase is increased, as well as the antitryptic value. At the end of the fourth week the lipoclastic acceleration had returned to normal and the antitryptic action to below normal. Similar observations were made after inoculation with normal rat tissue. The acceleration is not so marked as in mice 'negative' to inoculation with mouse tumour, but it may be observed that the accelerating action of the serum in normal rats is much higher than that of the serum of normal mice.

Up to this point, the reactions I have described refer to mice and rats which had each received one inoculation.

The present observations refer to the reactions of serum in repeated inoculation of cancer of a strange species.

[1] *Berl. klin. Wochenschr.*, 1908, xiv. 689. [2] *Ib.*, p. 1041.

[3] *Proceedings of Roy. Soc. Med.* (Therap. and Pharm. Sect.), 1912, v. 152-61, and 1913, vi. 114-18.

[4] *Proceedings of the Physiological Society*, January, 1911; *Journal of Physiology*, 1911, xlii, p. xi.

[5] C. Mackay, *Brit. Med. Journ.*, 1907, ii. 138.

In July, 1912, Dr. G. Carrington Purvis, now Medical Officer of Health, Grahamstown, who was home from South Africa, gave me some serum which he had obtained, some two years previously, after several inoculations of human cancerous material in a sheep. At that time he sent home to the Scottish Microscopical Society slides of some fungoid growths which he had obtained from human cancerous tissues by certain cultural methods, namely, by placing pieces of cancerous tissues in sodium salicylate and saline solution.[1]

He had proceeded to inoculate the sheep with these fungoid growths and the cancerous tissues (scirrhus and epithelioma) in which these vegetable growths were developing. His object was to prepare a cytolytic serum, or a serum which would kill the cells of cancer occurring in a human being.[2]

My examination of the solution in which mouse tumour had been recently kept by Dr. Purvis showed that the fluid increased the action of pancreatic lipase more than sodium salicylate and saline did alone or in combination; that is to say, the cancerous tissue had imparted some of its activating properties to the solution; no antitryptic action was exerted by the solution or by sodium salicylate and saline.

The experiment detailed in Table II shows the results I obtained on examination of the inoculated sheep serum. This experiment was repeated four times with similar results, namely (*a*) the lipoclastic accelerating action of the serum of the inoculated sheep was somewhat reduced as compared with normal fresh sheep serum; (*b*) the antitryptic action was completely absent. The inoculated sheep serum was, however, old, preserved in the usual way with antiseptic, and therefore no conclusion could be arrived at.

It corresponded, nevertheless, in both reactions, to what I had observed previously in therapeutic preparations of antidiphtheritic serum and of other antitoxic sera and vaccines. Tuberculin, for instance, gives a low lipoclastic acceleration, while the antitryptic action is absent. Similar reactions are found with calf vaccine lymph, the material for which examination I am indebted to Dr. A. B. Green. In this category perhaps I may include the serum of pregnancy, similar reduced reactions having been found in this condition in the rabbit and guinea-pig. In human pregnancy, however, the antitryptic action of the serum is increased (Table III).

In fresh antitoxic diphtheria serum the lipoclastic acceleration is somewhat reduced as compared with normal fresh horse serum; the antitryptic action is present, but not increased. For these specimens I am indebted to Dr. H. H. Dale.

I am still further indebted to Dr. J. Biernacki and Dr. A. L. Dykes for

[1] According to Dr. Purvis's method, small pieces of cancerous tissue (about $\frac{1}{4}$ inch cube) are placed in flasks containing about 30 c.c. of a one per cent. solution of sodium salicylate in 0·6–0·7 saline, about three pieces to each flask, and the tissue should remain in the solution from a week or ten days to four or six weeks, before inoculation. One piece with about 7 c.c. of the solution is used for each inoculation.

[2] G. C. Purvis, *Lancet*, 1912, ii. 438, and *Proceedings Scottish Micr. Soc.*, 1912, vol. vi, p. 43.

the opportunity of examining the blood in a case of diphtheria before treatment, and in a case after antitoxin treatment.

In the untreated case the lipoclastic acceleration is low and the antitryptic somewhat increased. In the treated case the lipoclastic acceleration is high and the antitryptic action reduced.

Table IV shows these results when the serum was examined in a similar way to that in Table I.

The necessity of repeating the experiments with fresh serum in animals repeatedly inoculated with cancer material or tissue was obvious.

With the kind assistance of Professor W. T. Hewlett, this I have done with human cancerous tissue (scirrhus of breast) in goat and sheep; and with mouse tumour in rats.

I am indebted to Dr. Purvis not only for the suggestion of repeated inoculation but for the information regarding his method and solution.

In these preliminary experiments, however, I have limited myself to human scirrhus of breast, and I have used fresh material as far as possible. I am indebted especially to Mr. D'Arcy Power for material, but in spite of his kindness and of others, relays of fresh scirrhus were not always available. I have used therefore in part material kept in Dr. Purvis's solution; in the case of the mouse tumour inoculation in rats, I have used fresh tissue; and in all cases inoculations were made subcutaneously, at intervals of a week.

Briefly summarizing, I found:

1. The grafts did not grow.

2. In the goat and rat some increase in the lipoclastic and antitryptic reactions of the serum were found after the first inoculation. In the sheep there was a drop in the lipoclastic reaction after the first inoculation, while the antitryptic remained the same. This, however, requires confirmation.

3. Ultimately, in all a point was reached of some reduction of the lipoclastic acceleration, while the antitryptic action was reduced to below normal. The response was found to vary in the different animals, for it required six inoculations in the goat and rats to produce a reduction in both reactions, whereas this was observed after two inoculations in the sheep.

4. In the same way similar reactions have followed repeated inoculations of carcinomatous serum, as suggested further by Dr. Purvis.

The results obtained are given in Table V as they were noted at the time.

Remarks. These reactions of the blood after cancer inoculation in animals, so far as they go, seem to show an analogy with other forms of immunity.

The reactions of the blood after repeated inoculation run parallel with the reactions in antidiphtheritic serum, other antitoxic sera and vaccines.

I have desired in the foregoing paper to place upon record some of the results I have obtained in my work, and in that of Dr. Purvis, rather than to attempt to draw any conclusions. In the present stage of inquiry definite conclusions are obviously impossible.

If, however, the analogy of the reactions I have described following inoculation with prepared or fresh cancerous tissue is admitted, this, it will be gathered, does not necessarily commit one to a parasitic theory.

For my part I have regarded the reactions as tissue or chemical reactions, supported as this view is by similar initial reactions in mice and rats after one inoculation of fresh normal tissues, tissue extracts and substances.

The more immediate point is whether serum obtained by Dr. Purvis's method, or by repeated inoculations of fresh human cancer tissue or serum in animals, possesses any therapeutic action.

So far, in a case recorded by Dr. Purvis, which he treated with the inoculated sheep serum, he thought he obtained necrosis in a large superficial epithelioma of the face. The condition of the patient was too advanced to form any decided conclusion.

In a recent letter Dr. Purvis mentions improvement in a laryngeal case being treated in this way when last seen.

Mr. Shattock has kindly told me that the result was negative in an experiment he performed originally on similar lines. In this instance he inoculated mouse tumour some eight times in a rabbit, using the serum of the rabbit on other tumour mice.

This negative result raises a question, namely, of suitability of animal, for so far as I have gone the reactions of the serum in the rabbit are variable after inoculation with vaccines, carcinomatous tissue and serum (human).

It is this point, together with the further questions of preparation of material, repeated inoculation, and reactions of the serum, which possibly may present fresh features for consideration.

I would take this opportunity of expressing my grateful thanks to Professor Halliburton for the facilities he has so freely afforded me in the Physiological Laboratory of King's College, and to many who have assisted me with material in this inquiry and in other ways.

TABLE I

Normal serum (a)

The accelerating action of normal human serum on pancreatic lipase varies considerably in health. It is usually higher during digestion, in trivial ailments, and for other unknown causes.

The *technique* has been more fully described in previous papers.

0·7 c.c. of diluted glycerin pancreatic extract (1–2) and 2·5 c.c. of olive oil emulsion were mixed, and after incubation the amount of fatty acids liberated required 10·9 c.c. of decinormal potash to neutralize it.

When 0·1 c.c. of serum from a healthy adult, collected one and a half hours after breakfast, had been added to the extract, this figure rose to 13·7; when 0·25 c.c. serum was added it increased to 16·6; and when 0·5 c.c. was added it rose to 18·6—that is, an increase of 7·7 for 0·5 c.c. serum.

In other cases it has been as low as 4–5, or as high as 10–13 for 0·5 c.c. of added serum.

The antitryptic value of the serum was 4–1 (plate method); that is, one part of serum inhibits digestion on serum plates of four parts of a one per cent. solution of trypsin.

Serum in carcinoma, sarcoma, and rodent ulcer (b)

In the same way, when, at the same time and under the same conditions, 0·1 c.c. of serum from a case of carcinoma of the stomach was added to the extract, the figure rose

to 16·6; when 0·25 c.c. serum was added it increased to 19·6; and when 0·5 c.c. was added it rose to 24·0—that is, an increase of 13·1 for 0·5 c.c. serum.

The antitryptic value of the serum was higher than 8–1.

Or in tabular form:

	c.c.	c.c. N/10 KOH required.	Acceleration.	Antitryptic.
Pancreatic extract alone on olive oil emulsion	...	10·9		
Normal addition of serum to extract	0·1	13·7	+ 2·8	4–1
	0·25	16·6	+ 5·7	
	0·5	18·6	+ 7·7	
Carcinoma of stomach ,, ,,	,,	16·6	+ 5·7	more than 8–1
	,,	19·6	+ 8·7	
	,,	24·0	+ 13·1	
Carcinoma of liver ,, ,,	,,	... ...	+ 8·4	more than 8–1
	,,	... ...	+ 13·1	
	,,	... ...	+ 15·1	
Carcinoma of rectum ,, ,,	,,	... ...	+ 4·6	more than 8–1
	,,	... ...	+ 8·9	
	,,	... ...	+ 9·7	
Carcinoma of breast; pleuritic fluid addition of fluid to extract	,,	... ...	+ 6·2	more than 8–1
	,,	... ...	+ 13·6	
	,,	... ...	+ 14·7	
Sarcoma addition of serum to extract	,,	... ...	+ 5·2	more than 8–1
	,,	... ...	+ 11·6	
	,,	... ...	+ 14·0	
Rodent ulcer ,, ,,	,,	... ...	+ 3·7	more than 10–1
	,,	... ...	+ 5·8	
	,,	... ...	+ 9·1	
Before and after treatment.				
Inoperable breast cancer ,, ,,	,,	... ...	+ 3·5	7–1
	,,	... ...	+ 7·8	
	,,	... ...	+ 8·6	
Apparent recovery ,, ,,	,,	... ...	+ 6·2	4–1
	,,	... ...	+ 11·3	
	,,	... ...	+ 14·7	
Inoperable uterine cancer ,, ,,	,,	... ...	+ 3·8	6–1
	,,	... ...	+ 5·5	
	,,	... ...	+ 10·1	
Apparent recovery ,, ,,	,,	... ...	+ 6·0	3–1
	,,	... ...	+ 11·1	
	,,	... ...	+ 14·2	

TABLE II

Serum of sheep repeatedly inoculated with human scirrhus and epithelioma (Dr. Purvis)

	Normal sheep serum.	*Serum of inoculated sheep.*	c.c. N/10 KOH required.	Acceleration.	Antitryptic.
Pancreatic extract alone on olive oil emulsion	... ...	... ...	10·0		
Addition of serum	0·1 c.c.	... ...	15·8	+ 5·8	4–1
	0·25 ,,	... ...	20·9	+ 10·9	
	0·5 ,,	... ...	23·9	+ 13·7	
		0·1 c.c.	15·8	+ 5·8	complete absence
		0·25 ,,	18·6	+ 8·6	
		0·5 ,,	22·6	+ 12·6	

TABLE III

Serum in pregnancy

	Normal serum control.	*Pregnancy near term.*	*Fœtal.*	*Acceleration.*	*Antitryptic.*
Human Addition of serum	0·1 c.c.	... ...	... ...	+ 5·9	4–1
	0·25 ,,	... ...	... ...	+ 9·4	
	0·5 ,,	... ...	... ...	+ 12·5	
		0·1 c.c.	... ...	+ 5·9	7–1
		0·25 ,,	... ...	+ 7·0	
		0·5 ,,	... ...	+ 10·3	
Guinea-pig Addition of serum	0·1 ,,	... ...	... ...	+ 7·2	5–1
	0·25 ,,	... ...	... ...	+ 9·9	
	0·5 ,,	... ...	... ...	+ 13·2	
		0·1 ,,	... ...	+ 6·1	3–1
		0·25 ,,	... ...	+ 7·7	
		0·5 ,,	... ...	+ 10·5	
			0·1 c.c.	+ 3·9	complete absence
			0·25 ,,	+ 5·0	
			0·5 ,,	+ 6·1	
		Tuberculin.			
Addition of vaccine	,,	... ...	... ...	+ 5·3	complete absence
				+ 7·8	
				+ 8·8	
		Calf Lymph.			
,,	,,	... ...	... ...	+ 7·0	complete absence
				+ 9·8	
				+ 10·9	

TABLE IV

Antidiphtheritic serum (a) *therapeutic* (*kept*)

	Normal horse serum.	*Antidiphtheritic serum.*	*Acceleration.*	*Antitryptic.*
Addition of serum	0·1 c.c.	... ...	+ 4·1	4–1
	0·25 ,,	... ...	+ 10·6	
	0·5 ,,	... ...	+ 12·7	
		0·1 c.c.	+ 4·0	complete absence
	... ...	0·25 ,,	+ 7·5	
		0·5 ,,	+ 11·7	
	(*b*) fresh			
,,	0·1 c.c.	... ...	+ 4·9	3–1
	0·25 ,,	... ...	+ 10·8	
	0·5 ,,	... ...	+ 14·2	
		0·1 c.c.	+ 3·9	3–1
		0·25 ,,	+ 9·3	
		0·5 ,,	+ 12·7	
		Diphtheria.		
,,	0·1 c.c.	... ...	+ 5·8	5–1
	0·25 ,,	... ...	+ 7·3	
	0·5 ,,	... ...	+ 9·3	
		Diphtheria, after antitoxin treatment.		
,,	,,	... ...	+ 5·5	3–1
	,,	... ...	+ 8·8	
	,,	... ...	+ 12·7	

TABLE V (*a*)

Serum of goat repeatedly inoculated with human scirrhus (breast)

Addition of serum.	*Normal before inoculation.*	*After inoculation 1 week.*	2 *weeks.*	3 *weeks.*	4 *weeks.*	5 *weeks.*	6 *weeks.*	*Antitryptic*
0·1 c.c.	+ 7·7							5-1
0·25 ,,	+ 12·2							
0·5 ,,	+ 14·5							
		+ 7·2						6-1
		+ 12·8						
		+ 16·0						
			+ 7·4					6-1
			+ 11·1					
			+ 15·4					
				+ 5·7				4-1
				+ 9·7				
				+ 11·6				
					+ 4·7			5-1
					+ 9·6			
					+ 11·6			
						+ 7·0		5-1
						+ 11·9		
						+ 13·3		
							+ 4·4	3-1
							+ 8·8	
							+ 11·2	

TABLE V (*b*)

Serum of rats repeatedly inoculated with mouse tumour

Addition of serum.	*Normal.*	*After inoculation 1 week.*	2 *weeks.*	3 *weeks.*	4 *weeks.*	5 *weeks.*	6 *weeks.*	7 *weeks.*	*Antitryptic.*
Example 1									
0·1 c.c.	+ 5·0								5-1
0·25 ,,	+ 9·8								
0·5 ,,	+ 13·1								
Example 2									
0·1 c.c.	+ 8·1								5-1
0·25 ,,	+ 13·6								
0·5 ,,	+ 14·7								
		+ 7·2							7-1
		+ 12·6							
		+ 15·5							
			+ 5·7						7-1
			+ 11·5						
			+ 14·7						
				+ 6·0					6-1
				+ 10·9					
				+ 14·4					
					+ 6·3				5-1
					+ 10·7				
					+ 13·2				
						+ 7·3			7-1
						+ 11·7			
						+ 13·7			
							+ —		3-1
							+ 9·4		
							+ 11·8		
								+ 6·3	3-1
								+ 10·4	
								+ 14·4	

TABLE V (*c*)

Serum of sheep repeatedly inoculated with human scirrhus (breast)

Addition of serum.	*Normal.*	*After inoculation 1 week.*	*2 weeks.*	*3 weeks.*	*4 weeks.*	*5 weeks.*	*Anti-tryptic.*
0·1 c.c.	+ 5·3						5–1
0·25 ,,	+ 8·4						
0·5 ,,	+ 13·1						
		+ 4·4					5–1
		+ 7·3					
		+ 10·7					
			+ 3·4				3–1
			+ 8·3				
			+ 11·7				
				+ 4·3			2–1
				+ 7·3			
				+ 11·1			
	Normal control.						
	+ 5·4						5–1
	+ 10·3						
	+ 13·2						
					+ 4·4		2–1
					+ 9·3		
					+ 11·7		
						+ 4·9	2–1
						+ 8·3	
						+ 11·7	

TABLE V (*d*)

Serum of sheep inoculated repeatedly with serum from case of inoperable rectal carcinoma—2·0 c.c. serum at intervals of one week

Addition of serum.	*Normal.*	*After inoculation 1 week.*	*2 weeks.*	*3 weeks.*	*4 weeks.*	*5 weeks.*	*Anti-tryptic.*
0·1 c.c.	+ 3·6						5–1
0·25 ,,	+ 7·3						
0·5 ,,	+ 12·4						
		+ 4·0					5–1
		+ 10·3					
		+ 15·0					
	Normal control.						
	+ 2·5						5–1
	+ 7·2						
	+ 13·0						
			+ 1·5				3–1
			+ 5·3				
			+ 10·9				
				+ 1·5			3–1
				+ 5·4			
				+ 10·9			
					+ 2·1		3–1
					+ 5·2		
					+ 9·2		
	Normal control.						
	+ 3·7						4–1
	+ 7·6						
	+ 13·1						
						+ 2·6	3–1
						+ 3·7	
						+ 9·8	

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